Co-infections with hepatitis B and C viruses in human immunodeficiency virus-infected patients in Morocco

K. Rebbani[1], A. Ouladlahsen[2], A. Bensghir[2], A. Akil[1], H. Lamdini[2], H. Issouf[1], I. Brahim[1], B. Kitab[1], F. Z. Fakhir[1], L. Wakrim[1], K. Marhoum El Filali[2], H. Himmich[2], S. Ezzikouri[1] and S. Benjelloun[1]

1) *Virology Unit, Viral Hepatitis Laboratory, Pasteur Institute of Morocco, Casablanca, Morocco and* 2) *Service des maladies infectieuses, CHU Ibn Rochd, Casablanca, Morocco*

Abstract

Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are major public health concerns. We aimed to determine the prevalence of HBV and HCV infections among HIV-infected patients, and to identify the main circulating hepatitis strains in Morocco. The study was carried out in 503 HIV-infected patients. Our survey indicated that the prevalence of HIV/hepatitis co-infection was 10.6%; 5.2% of patients were HBV surface antigen positive, and 5.4% of patients were anti-HCV positive. Among the HBV surface antigen-positive group, HBV DNA sequencing identified exclusively genotype D (D1: 26.7%; D7: 73.3%) in accordance with what is found in the general population. In contrast, sequencing of HCV isolates produced an unusual subtype distribution with a decreasing order of prevalence: 1a, 3a (both 23.5%), 1b, 4a (both 17.6%), 1c (11.8%) and 6h (6%).

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Corresponding author: S. Benjelloun, Virology Unit, Viral Hepatitis Laboratory, Institut Pasteur du Maroc 1, Place Louis Pasteur, 20360 Casablanca, Morocco
E-mail: soumaya.benjelloun@pasteur.ma

Introduction

Infections with human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) rank among the ten leading causes of death from infectious diseases [1].

Current data show that the variability in the prevalence of HIV/hepatitis co-infection worldwide is multifactorial, and depends on the geographic regions, the infection risk factors, and the type of exposure [2,3]. In Morocco, the WHO estimated that in 2008, about 21 000 people were living with HIV infection [4].

To the best of our knowledge, HIV/hepatitis co-infection is still poorly documented and more information is needed to better understand the Moroccan context. The aim of the present study was, therefore, to characterize epidemiological and molecular aspects of HIV/hepatitis co-infection in a Moroccan cohort of HIV-infected patients.

This study included a cohort of 503 HIV-infected patients (245 men and 258 women) followed for treatment in the Infectious Disease Centre, CHU Ibn Rochd, Casablanca from January 2006 to June 2010. Sociodemographic parameters and clinical data (disease diagnosis, antiretroviral treatment, laboratory values, alcohol consumption and drug use history) were extracted in detail from the department clinical database Nadis® [5]. The study was approved by the ethics committee of Institut Pasteur of Morocco, and privacy and confidentiality conditions were guaranteed.

For all patients with HIV infection, serological markers for HBV (HBV surface antigen (HBsAg), HBV e-antigen (HBeAg), and antibodies to HB surface antigen, core antigen and e-antigen (anti-HBs, anti-HBc, anti-HBe)) and HCV (anti-HCV antibodies) were tested with commercially available kits (AxSYM; Abbott Laboratories, Wiesbaden-Delkenheim, Germany), and for hepatitis D virus (IgG-Ab) using ETI-AB-DELTAK-2 (DiaSorin S.P.A, Saluggia, Italy). Positive samples for HBsAg and anti-HCV antibodies were made available for further molecular analysis (PCR, reverse transcription-PCR and genotyping).

HBV DNA and HCV RNA were isolated using the QIAamp Viral extraction Kit (QIAgen,Valencia, CA, USA), according to the manufacturer's instructions.

Plasma HCV RNA and HBV DNA were quantified by real-time PCR using Syber® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on a thermocycling machine according to the manufacturer's instructions (ABI PRISM 7000; Applied Biosystems).

For genotyping, the HBV surface (S) gene was amplified as previously described [6]. HCV RNA was isolated and cDNA was synthesized using the Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) (Invitrogen, Saint Aubin, France), according to the manufacturers' instructions, and then amplification was carried out using primers matching conserved regions in the 5′-untranslated region and NS5B using conditions previously described [7,8].

Positive PCR products were purified using the Exonuclease I/Shrimp Alkaline Phosphatase (GE Healthcare, Chalfont St Giles, UK) and bidirectionally sequenced using BigDye Terminator version 3.1 kits and an ABI PRISM 3130 DNA automated sequencer (Applied Biosystems). Sequence analysis was performed with SeqScape® v2.5 software (Applied Biosystems) by comparing the study sequences to a set of reference sequences using BLAST.

Results were reported as mean and standard deviation or median and range for continuous variables, and frequency counts for categorical variables. Categorical variables were evaluated using chi-squared or Fisher's exact test. One-way analysis of variance was conducted to compare mean quantitative values. All p-values were two sided, and differences with $p < 0.05$ were considered to be statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA).

Baseline characteristics of the patients are described in Table 1. Patients were grouped into HIV/HBV co-infected, HIV/HCV co-infected, and HIV mono-infected (HIV+) patients, according to serological and molecular data as described below (Table 2).

From 503 HIV-positive patients, 148 (29.4%) were found to be anti-HBc positive, 93 (18.5%) had anti-HBs (nine patients with only anti-HBs and 84 patients with both anti-HBs and anti-HBc), whereas 26 (5.2%) were positive for HBsAg (Table 2). Anti-delta IgG-Ab was sought in all HBV-infected patients, but none was found. HBV DNA was undetectable for 11 patients (42.3%), but the remaining HBsAg(+) patients had detectable DNA (15/26, 57.7%). Sequencing and phylogenetic analysis of the surface gene indicated that HBV strains belonged exclusively to genotype D (D7: 73.3%, D1: 26.7%).

Twenty-seven cases (5.4%) within the cohort studied had anti-HCV antibodies, and viral RNA load was subsequently measured in these patients. HCV RNA was undetectable for eight patients (29.6%). Among the 19 (70.4%) HIV/HCV co-infected patients positive for HCV RNA, 17 were successfully sequenced, and genotype 1 (9/17, 52.9%) was present in the majority of cases, but distributed among three subtypes (1a, 23.5%; 1b, 17.6%; 1c, 11.8%). Sizeable subsets of HCV strains were composed of 3a and 4a subtypes (23.5% and 17.6%, respectively).

According to the CDC staging system of HIV disease, patients were classified as CDC (A/B) and CDC (C) stages. Interestingly, we found a significant association between HIV/hepatitis co-infection, and advanced HIV disease stage; 39.7% of HIV/hepatitis patients versus 26.7% of HIV+ only patients (p 0.0187) had CDC (C) stage disease. Among the HBV-DNA-positive patients and HCV-RNA-positive group, 61% and 41% had CDC (C) stage disease, respectively.

TABLE 1. Characteristics of human immunodeficiency virus (HIV) -infected patient's cohort at the time of enrolment

	HIV mono-infected	HIV/Hepatitis co-infected	p
Total	450	53	
Age (mean ± SD), year	38 ± 9	40 ± 7	0.048
Sex			
Male	203	42	2.0E-6
Female	247	11	
Ethnic groups			
Moroccan	442	52	0.393
Foreigners living in Morocco	8	1	
Transmission route			
Sexual contact	420	38	1.0E-5
Injecting drug use	5	7	4.0E-5
Pregnancy	3	0	0.715
Blood transfusion	2	0	0.800
Others	20	8	0.004
Antiretroviral therapy			
Yes	350	45	
No	100	8	0.073
CD4 cell count (cells/mm^3)			
<350	291	36	
>350	159	17	0.097
CDC Stage			
A/B	330	32	0.018
C	120	21	
HIV load			
<1000 copies/mL	112	21	0.019
>1000 copies/mL	307	32	

TABLE 2. Molecular, clinicopathological parameters and seroprevalence of human immunodeficiency virus (HIV)/hepatitis B virus (HBV) and HIV/hepatitis C virus (HCV) co-infected patients

	Co-infected B (HBsAg+) *n* = 26	Co-infected C (HCV Ab+) *n* = 27
Serological markers		
Anti-HBs	0	8 (29.6%)
Anti-HBc	26 (100%)	14 (51.8%)
HBeAg	7 (26.9%)	0
Anti-HBe	17 (65.4%)	–
Age (mean ± SD), year	40 ± 7	
Sex		
Male	18 (69.2%)	24 (88.9%)
Female	8 (30.8%)	3 (11.1%)
Antiretroviral therapy		
Yes	25 (96%)	20 (74%)
No	1 (4%)	7 (26%)
CD4 cell count (cells/mm^3)		
<350	19 (73%)	17 (63%)
>350	7 (27%)	10 (37%)
CDC stage		
A/B	14 (53.8%)	18 (66.7%)
C	12 (46.2%)	9 (33.3%)
HIV load before co-infection (copies/mL)		
Low <1000 copies/mL	8 (30.8%)	13 (48.2%)
High >1000 copies/mL	18 (69.2%)	14 (51.8%)
AST (median ± SD), IU/L	48 ± 29	64 ± 62
ALT (median ± SD), IU/L	52 ± 43	76 ± 99
HBV DNA+	15 (57.7%)	–
HCV RNA+	–	19 (70.4%)
Median hepatitis viral load (copies/mL)	722.35 (0; 16 242 857)	11969 (0; 5 437 827)
Genotypes and subgenotypes	D7 (73.3%) D1 (26.7%)	1a (23.5%) 1b (17.6%) 1c (11.8%) 3a (23.5%) 4a (17.6%) 6 h (6%)

anti-HBc, anti-HBV core antigen; anti-HBe, anti-HBV e-antigen; anti-HBs, anti-HBV surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, HBV e-antigen.

Morocco is a country of intermediate endemicity for hepatitis B and C. A recent survey of HBsAg carriage reported a prevalence of 1.66% whereas another one showed an anti-HCV prevalence of 1.93% [9–11]. In the current study, we have shown that prevalence of HIV/hepatitis co-infection was 5.2% for HIV/HBV and 5.4% for HIV/HCV within the Moroccan HIV-infected population. This indicated that HIV-infected patients form a high-risk group for HBV and HCV co-infection compared with the general population. This is probably because the three viruses share similar routes of transmission [2].

Co-infection with HIV/hepatitis in the North African context is still poorly studied. The prevalence of co-infection with HCV has been reported to be variable, and dependent on the route of transmission [2].

In Tunisia, a prevalence of 40% anti-HCV positivity has been reported among HIV-infected patients, of whom 78% were injecting drug users [12,13]. In Algeria, the prevalence of anti-HCV among HIV-infected patients has been measured to be 6% [14]. In Morocco, Benjelloun *et al.* [15] reported that the prevalence of HIV/HCV co-infections two decades ago was approximately 20%. The discordance of these results and the present report can be explained by the fact that the HIV-positive group studied in 1996 were mostly injecting drug users or professional sex workers. Such populations were obviously at high risk for additional viral infections among which hepatitis C is prominent [16]. In the present study, the group surveyed was a component of the general population, and not just a subset of high-risk individuals. Hence, these patients had different socio-economic backgrounds, and accordingly the main route of HIV infection was heterosexual contact. In fact, of the 503 patients included in this study, only 12 patients had a history of injection drug use, of whom seven patients were found to be anti-HCV positive. Finally, recent informational campaigns (since 2004) have increased Moroccan public awareness about preventive measures against infection with HIV. Hence, it is hypothesized that such policies have already had an impact in reducing the incidence of HIV/HCV co-infections in the Moroccan population.

As in all Mediterranean countries, the genotype D is the predominant HBV genotype in the Moroccan population [6]. The same also holds true in HIV-infected patients. Most genotype D strains are classified as D7 subtype (73.3%), whereas the remaining 26.7% are subtype D1. This result is consistent with earlier studies from the Maghreb [6,17]. With regards to HIV/HCV co-infection, our results showed that subtype 1b isolates are closely related to the previously published Maghrebian and European strains. In HIV(+) patients, subtype 1b is no longer the predominant genotype as has been reported in HIV(−) Moroccan patients with chronic hepatitis C (67.5%) or HCV-associated hepatocellular carcinoma (84.4%) [7]. Additional phyla of genotype 1 (subtypes 1a and 1c) are also present in co-infected patients whereas they are almost absent from HIV(−) populations. In contrast to what has been observed in the general population where the ancestral West African genotype 2 closely follows subtype 1b, we observed striking prevalences of subtypes 3a and 4a (23.5% and 17.6%, respectively) in co-infected patients [7]. Such HCV subtype distributions in the HIV/HCV co-infected population was somewhat unexpected. This result shows that the epidemiology of hepatitis virus strains circulating among HIV (+) patients is much more complex than what we observed in HIV(−) patients. Such diversity could be reasonably explained by the geographical, familial and economic connections developed between Morocco and Europe (3a), the Middle East (4a), and in particular countries like France, Italy, Egypt and Saudi Arabia. A similar situation was not found in the case of the less diverse HBV isolates for which genotype D has been found to be predominant from Morocco to India.

In conclusion, the endemicities of chronic hepatitis viral infections in Morocco put HIV-infected patients at high risk for co-infection with these agents. The current study provides an epidemiological overview of the HIV/hepatitis co-infection situation in the country, and emphasizes the urgent need for the rapid implementation of more efficient preventive and monitoring programmes.

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Transparency Declaration

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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